ORIGINAL ARTICLE

A Genome-Wide Association Study Identifies *GRK5* and *RASGRP1* as Type 2 Diabetes Loci in Chinese Hans

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Substantial progress has been made in identification of type 2 diabetes (T2D) risk loci in the past few years, but our understanding of the genetic basis of T2D in ethnically diverse populations remains limited. We performed a genome-wide association study and a replication study in Chinese Hans comprising 8,569 T2D case subjects and 8,923 control subjects in total, from which 10 single nucleotide polymorphisms were selected for further follow-up in a de novo replication sample of 3,410 T2D case and 3,412 control subjects and an in silico replication sample of 6,952 T2D case and 11,865 control subjects. Besides confirming seven established T2D loci (*CDKAL1*, *CDKN2A/B*, *KCNQ1*, *CDC123*, *GLIS3*, *HNF1B*, and *DUSP9*) at genome-wide significance, we identified two novel T2D loci, including G-protein–coupled receptor kinase 5 (*GRK5*) (rs10886471: $P = 7.1 \times 10^{-9}$) and *RASGRP1* (rs7403531: $P = 3.9 \times 10^{-9}$), of which the association signal at *GRK5* seems to be specific to East Asians. In nondiabetic individuals, the T2D risk-increasing allele of *RASGRP1*-rs7403531 was also associated with higher HbA_{1c} and lower homeostasis model assessment of β-cell function ($P = 0.03$ and 0.0209, respectively), whereas the T2D risk-increasing allele of *GRK5*-rs10886471 was also associated with higher fasting insulin ($P = 0.0169$) but not with fasting glucose. Our findings not only provide new insights into the pathophysiology of T2D, but may also shed light on the ethnic differences in T2D susceptibility. ***Diabetes* 62:291–298, 2013**

The prevalence of type 2 diabetes (T2D) has increased dramatically in China during the past few decades (1), and currently >92 million Chinese adults are estimated to have T2D (2). Although nutritional transition, lifestyle changes, and increasing obesity prevalence are important risk factors driving the epidemic in China, genetic factors also play a major role in T2D susceptibility (3,4). Genome-wide association studies (GWAS) have identified >50 T2D susceptibility loci, predominantly in populations of European ancestry, but also in East and South Asians (5). Risk variants at these loci are generally of modest effect and altogether explain only 10–15% of the heritability of T2D (6). East Asians, including Chinese, have been shown to be genetically more susceptible to developing T2D than Western populations (7,8), but the genetic mechanism underlying this ethnic difference remains poorly understood (9). Although at least 14 T2D loci have been identified through GWAS in East Asian populations (5,10–12), these loci are not sufficient to explain the ethnic difference in T2D susceptibility. Moreover, some of these loci remain to be validated in additional independent cohorts. In this study, we describe a three-stage GWAS of T2D in Chinese Hans that aims to identify additional T2D susceptibility loci (Fig. 1).

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*A complete list of the DIAGRAM Consortium and AGEN-T2D Consortium is provided in the Supplementary Data.

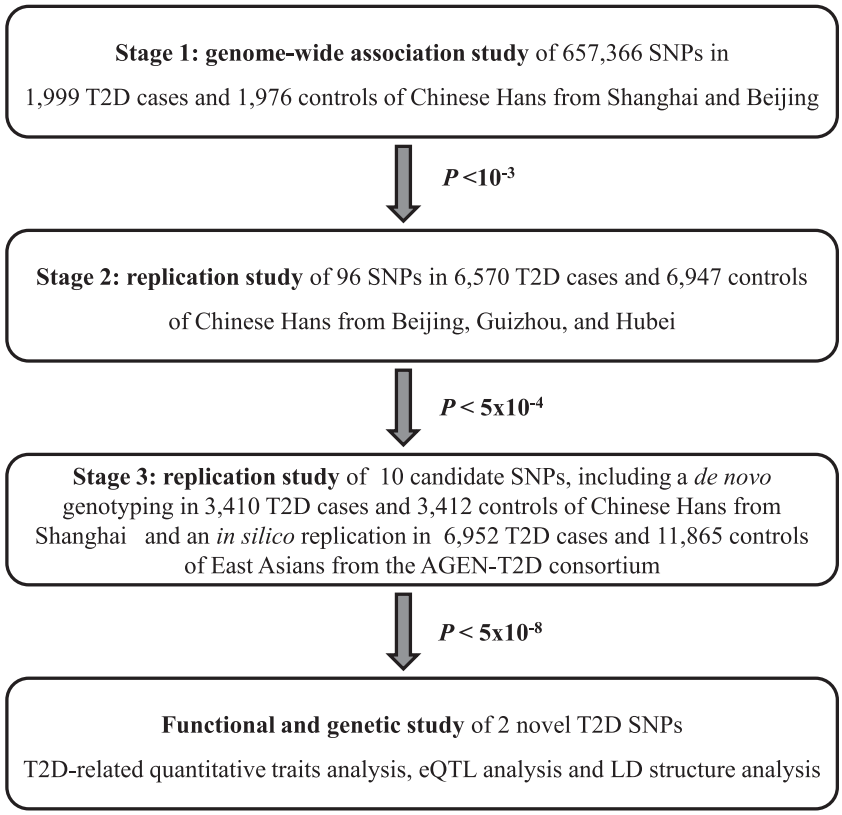

FIG. 1. Summary of study design.

RESEARCH DESIGN AND METHODS

Participants. We performed a three-stage GWAS in multiple independent sample sets. The stage 1 samples for the GWA scan included 1,999 T2D case subjects and 1,976 nondiabetic control subjects drawn from the Nutrition and Health of Aging Population in China (NHAPC) study (312 case and 815 control subjects), the Gut Microbiota and Obesity Study (GMOS) (82 case and 163 control subjects), the Fudan-Huashan Study (807 case and 339 control subjects), and the Beijing Diabetes Survey (798 case and 659 control subjects). The stage 2 samples for replication testing of 96 single nucleotide polymorphisms (SNPs) consisted of 13,517 unrelated Chinese Hans (6,570 T2D case and 6,947 nondiabetic control subjects) from the Guizhou-Bijie Type 2 Diabetes Study (GBTDS), the Beijing Type 2 Diabetes Studies (BTDS), and the Hubei Type 2 Diabetes Studies (HTDS). The stage 3 replication testing of 10 SNPs is carried out by de novo genotyping in 3,410 T2D patients and 3,412 nondiabetic control subjects from the Shanghai Diabetes Inpatient Database (SDIID) and the Shanghai Diabetes Study (SDS) and in silico replication in the AGEN-T2D GWAS datasets (6,952 T2D case and 11,865 control subjects). There was no overlap of participants among stage 1, 2, and 3 samples. Studies of T2D-related quantitative traits were performed among 3,614 participants from the NHAPC and GMOS, the two population-based studies. All studies were approved by local ethics committees of each participating institution, and informed written consent was obtained from all participants. A summary of the contributing studies, criteria for T2D case and nondiabetic control subjects in each study, and sample characteristics can be found in the Supplementary Materials and Methods and Supplementary Table 1.

Genotyping and quality control. The stage 1 DNA samples were genotyped using the Illumina Human660W-Quad BeadChip (Illumina, Inc., San Diego, CA). Quality control (QC) filters were applied at the individual and SNP levels. At the individual level, we removed the samples that met any of the following criteria: *1*) call rates <97% (n = 20); *2*) with excessive heterozygosity; *3*) with sex mismatches between the reported and genetically inferred (n = 69); and *4*) with unexpected duplicates (n = 19) or cryptic relatives (n = 149). We also removed population outliers (n = 6) detected by using principle component analysis (13) (Supplementary Fig. 1). At the SNP level, we excluded the copy number variation–related SNPs, the SNPs in Y and mitochondrial chromosomes, and the SNPs if they had: 1) call rate <95% (n = 1,351); 2) minor allele frequency <0–5% (n = 63,263); and 3) Hardy-Weinberg equilibrium (HWE) $P < 10^{-6}$ in control groups (n = 1,047). The samples that passed all QC criteria were then used to impute for the ungenotyped or missing SNPs from the phase 2 HapMap CHB+JPT (release number 22) reference panel using IMPUTE (version 2.1.2; http://mathgen.stats.ox.ac.uk/impute/impute.html) software (14). We removed all imputed SNPs with an estimated call rate <99%, minor allele frequency <1%, HWE $P < 10^{-6}$, or the info measure ≤0.5.

For stage 2 replication, we genotyped 96 SNPs selected from stage 1 in three independent Chinese Han populations with 6,570 T2D case subjects and 6,947 control subjects from the GBTDS, BTDS, and HTDS studies using TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, CA) in the Fludigm EP1 platform. We excluded the samples with call rate <93% and the SNPs with call rate <95% or deviation from HWE at $P < 5.2 \times 10^{-4}$ (Bonferroni corrected P value for 96 tests) in control groups. The overall call rates were 99.7, 99.4, and 99.4% for samples from the GBTDS, BTDS, and HTDS studies, respectively. We genotyped >5% duplicate samples for each study to assess genotyping reproducibility, and the concordance rates were 99.9% for 189 duplicate samples from the GBTDS and 99.5% for 387 duplicate samples from BTDS and for 166 duplicate samples from HTDS. We also performed cross-platform validation by genotyping the 96 SNPs in 135 stage 1 samples that had been run on the Illumina assay using TaqMan SNP Genotyping Assays in Fludigm EP1 platform, and the concordance rate was 99.9%.

Finally, 10 SNPs, selected based on results of meta-analysis that combined stage 1 and 2 data, were used for stage 3 replication, including a de novo genotyping in 3,410 T2D case and 3,412 control subjects from the SDIID/SDS study and an in silico replication in 8 independent GWAS datasets of 6,952 case and 11,865 control subjects from the AGEN Consortium. The de novo genotyping was performed using Sequenom MassARRAY (Sequenom, San Diego, CA) in the SDIID/SDS study. After quality control, 3,257 T2D case and 3,262 control subjects with genotyping call rate >90% were used for association analyses. The concordant rates were >99% for all tested SNPs in 232 duplicated samples. The in silico replication results were obtained by directly searching the meta-analysis results of 8 independent GWAS datasets with 6,952 case and 11,865 control subjects in total that participated in the discovery stage of the AGEN-T2D Consortium.

Statistical analysis. For stage 1 GWAS, the genotyped SNPs (495,686) and imputed SNPs (1,738,508) were tested for their associations with T2D using PLINK (version 1.07; http://pngu.mgh.harvard.edu/~purcell/plink/) (15), and SNPTEST (version 2.2.0; https://mathgen.stats.ox.ac.uk/genetics_software/snptest/snptest.html) (14), respectively. The odds ratios (ORs) and 95% CIs

were estimated by logistic regression under an additive genetic model, adjusting for age, sex, and the first two principal components in model 1. BMI was further included as a covariate in model 2. For the X-chromosome SNPs, males were treated as homozygotes, in that genotypes were coded as 2 for the coded allele (16). The initial association analyses were performed by pooling the data from the Beijing and Shanghai participants and then stratified by geographical regions (Beijing and Shanghai) to test whether the pooling would introduce a bias due to potential population stratification. The *P* values were adjusted for genomic control inflation factor (λ_{GC}). To find more consistent evidence for associations between each SNPs and T2D, we further stratified the association analyses by sex or tested the associations between the case subjects with family history and the nondiabetic control subjects.

The SNP selection for stage 2 replication was largely based on the *P* values in the discovery stage, and one or two SNPs with the smallest *P* values were chosen for each significant genomic region, defined as that contained a set of SNPs in linkage disequilibrium (LD) at $r^2 \geq 0.1$ with the most associated SNP.

For stage 2 and 3 replication analyses, logistic regression analysis was applied to test association of each SNP with T2D, assuming an additive genetic effect and adjusting for age and sex. The association analyses were performed separately in each study. The effect sizes across studies or stages were combined using fixed-effect inverse variance weighted meta-analysis, and the *P* values were calculated using a fixed effects meta-analysis with sample size weighted *Z*-score. The heterogeneity across studies was assessed using Cochran Q statistics (17).

We also examined the effect of T2D-associated SNPs on T2D-related quantitative traits in participants from the NHAPC and GMOS studies, of which the individuals receiving glucose-lowering treatment and GMOS participants with BMI ≥28 were excluded from analyses (Supplementary Table 10). The NHAPC samples were genotyped using the Illumina Human660W-Quad BeadChip (Illumina, Inc.) using the stage 1 QC criteria, and the GMOS samples were genotyped using TaqMan SNP Genotyping Assays in Fludigm EP1 platform. Finally, a total of 3,614 samples (2,229 from Shanghai and 1,385 from Beijing) that passed all QC criteria were used for the association analyses, which were performed in the Shanghai and Beijing samples separately using linear regression analysis assuming an additive genetic model, adjusting for age, sex, and study covariates (NHAPC and GMOS for the Shanghai samples only). Values of insulin and homeostasis model assessment of β-cell function (HOMA-B) and insulin sensitivity (HOMA-S) were natural log-transformed before analysis, and results of each study were combined by fixed-effect inverse variance weighted meta-analysis. HOMA-S and HOMA-B were estimated by the HOMA model using Levy's computer model (18).

***cis*-eQTL and peripheral blood mRNA expression analyses.** For *cis*-eQTL analysis, we first searched rs10814916, rs10886471, rs7403531, and their proxy SNPs ($r^2 > 0.5$) in the Genevar eQTL database, including 166 adipose tissues, 156 lymphoblastoid cell lines, and 160 skin tissues derived from a subset of healthy female twins of the MuTHER resource (19). Associations between each SNP and mRNA expression level of nearby genes were obtained by using Spearman rank correlation for <10,000 permutations as the default settings of Genevar (version 3.1.1; http://www.sanger.ac.uk/resources/software/genevar/).

We performed *GKR5* mRNA expression analysis in blood samples of 64 unrelated Chinese Hans recruited from the Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, in December 2011. All participants provided written informed consent, and study protocol was approved by the local ethics committee. Genomic DNA and RNA samples were extracted from peripheral blood by using a Qiagen kit (Qiagen) and RNA Pure Blood kit (CoWin Biotech Co.), respectively. The genotype of G-protein–coupled receptor (GPCR) kinase 5 (*GRK5*)-rs10886471 was determined by sequencing, using the following primers: 5′-TCCTACACTGGAACAAGCC-3′ and 5′-ACGGACTA-ATACAGACGGG-3′. Quantitative real-time RT-PCR for *GRK5*-rs10886471 was performed using the GoldStar TaqMan Mixture kit (CoWin Biotech Co.) on a Bio-Rad IQ5 system (Bio-Rad). *GRK5* mRNA levels were normalized to the relative expression level of *GAPDH*. We carried out statistical analysis for expression data using linear regressions and an unpaired Student *t* test. *P* value from linear regression was calculated by assuming a dominant genetic effect of minor allele of rs10886471 and adjusted for the status of T2D. The one-tail *P* value is reported in the text.

Comparison of regional LD patterns. The genomic regions for the *GRK5* and *RASGRP1* loci were selected based on their regional LD plots (Supplementary Figs. 6 and 7). The varLD algorithm is used to compare difference in the regional LD patterns among phase 2 HapMap CHB samples (45 Chinese Hans), CEU samples (60 white Europeans), and JPT samples (45 Japanese), and results were presented as Monte Carlo *P* values (20).

RESULTS

In stage 1, we tested the association with T2D of 2,234,194 genotyped and imputed SNPs that passed all QC criteria in 3,712 Chinese Hans from Beijing and Shanghai, of which 1,839 were case subjects, and 1,873 were control subjects. No significant population stratification was observed between case and control subjects using principle component analysis (Supplementary Fig. 2), and the genomic control factor was also quite close to 1.0 (λ = 1.03), suggesting there is no genome-wide inflation due to population stratification for stage 1 samples. The quantile–quantile plot showed substantial deviation from the expected at the lower *P* values, suggesting that some associations were more significant than expected by chance (Supplementary Fig. 3). SNPs in four previously reported T2D loci (*CDKAL1*, *CDKN2A/B*, *KCNQ1*, and *DUSP9*) reached genome-wide significance ($P < 5 \times 10^{-8}$) (Fig. 2 and Supplementary Fig. 4), with directional consistency as previously reported (21–25). For the other previously reported T2D loci, apart from three loci that were monomorphic in Chinese Hans, 48 of 51 loci showed directionally consistent association with T2D (binomial test, $P = 9.8 \times 10^{-12}$), of which 19 were associated with T2D at $P < 0.05$ (Supplementary Table 3). Of these, only the rs7754840 in *CDKAL1* showed a significantly larger effect on T2D in Chinese Hans than in white Europeans (*P* for heterogeneity = 3.33×10^{-4}), consistent with our previous findings (26).

For follow-up, we chose one or two SNPs per locus that had the smallest *P* values in stage 1, except for the two strongest association signals at *CDKAL1* and *KCNQ1* ($P \leq 4.24 \times 10^{-10}$, OR = 1.37), for which association with T2D has been repeatedly confirmed in East Asian populations.

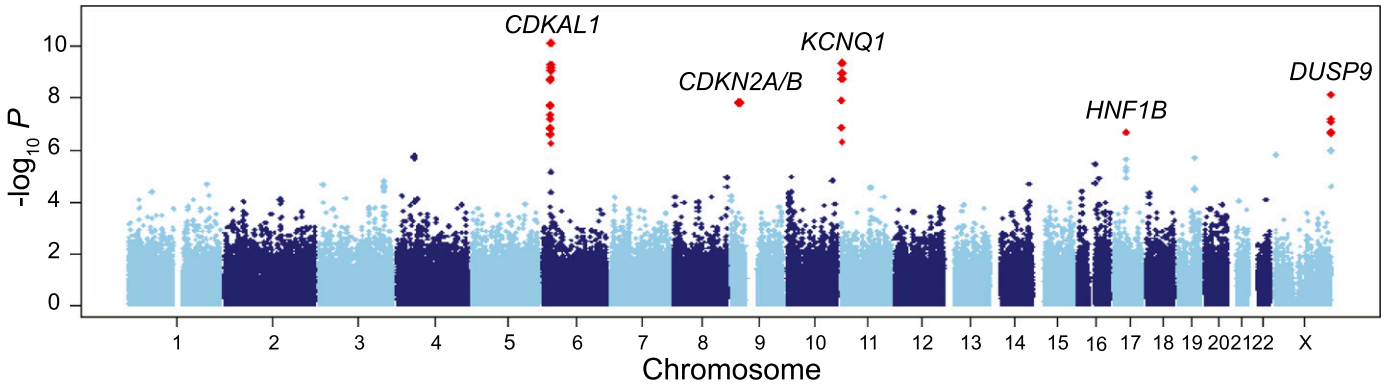

FIG. 2. Manhattan plot for genome-wide association analysis of 495,686 genotyped SNPs in stage 1. The $-\log_{10} P$ values were from pooled analysis, adjusting for age, sex, and the first two principle components. The red dots at each locus indicate the signals with $P < 10^{-6}$.

As such, the 96 most significant SNPs representing 84 independent loci were selected for stage 2 replication (Supplementary Tables 2 and 4), which consisted of de novo genotyping of the SNPs in three independent Chinese Han populations with 6,570 T2D case and 6,947 control subjects (Supplementary Materials and Methods and Supplementary Table 1). All SNPs, except for *SLC30A8*-rs13266634 that showed significant deviation from HWE, passed the QC criteria and were included in the replication analyses. A total of eight SNPs in or near *RASGRP1*, *GLIS3*, *CDKN2B*, *CDC123*, *HHEX*, *HNF1B*, *FAM58A*, and *DUSP9* were significantly associated with T2D after Bonferroni correction for multiple tests in the stage 2 samples, and the *P* value cutoff for Bonferroni correction was 5.26×10^{-4} (0.05/95) (Supplementary Table 5). In meta-analyses combining stage 1 and 2 data, the SNPs in or near *RASGRP1*, *CDKN2B*, *CDC123*, *HHEX*, *HNF1B*, *FAM58A*, and *DUSP9* reached the genome-wide significance (Supplementary Table 5), of which *CDKN2B* (24,25,27), *HHEX* (24,25,27), *CDC123* (28), *HNF1B* (29), and *DUSP9* (29) are established T2D loci. rs10814916 ($P = 5.29 \times 10^{-7}$) at *GLIS3* and rs10886471 ($P = 5.92 \times 10^{-7}$) at *GRK5* were close to the genome-wide significance threshold (Supplementary Table 5). The association between *GLIS3* variant (rs7034200) and T2D risk had been observed in a previous candidate gene association study (30), but the evidence was inconclusive. The SNP rs12010175 in *FAM58A* is in modest LD with rs5945326 near *DUSP9* ($r^2 = 0.35$). Conditional analyses by including the two SNPs in one logistic regression model revealed that these two SNPs represent the same locus (*P* adjusted for rs5945326 = 0.02).

Apart from the SNPs that represent previously established T2D loci, 10 SNPs (each representing independent loci) showed association with T2D at $P < 5 \times 10^{-4}$ in a meta-analysis that combined stage 1 and 2 data, and no additional novel signal ($P < 5 \times 10^{-4}$) was observed when the association analyses were stratified by sex (Supplementary Table 6). These 10 SNPs were taken forward for replication in stage 3, which included a de novo genotyping replication in 3,410 T2D case and 3,412 control subjects of Chinese Hans and an in silico replication in a previously published meta-analysis of GWAS of 6,952 T2D case and 11,865 control subjects (including 4,026 case and 4,654 control subjects from Chinese Hans and the remaining 2,926 case and 7,211 control subjects from Korean, Japanese, Malay, and Filipino populations) of the AGEN-T2D Consortium (5). A total of three SNPs at *GLIS3*, *GRK5*, and *RASGRP1*, respectively, exceeded the genome-wide significance threshold (combined *P* values: 7.1×10^{-9} to 6.0×10^{-12}; OR: 1.10–1.12) when we combined the data across all three stages (Table 1 and Supplementary Table 7). We found no evidence of heterogeneity for the associations at these three loci across all study populations (*P* for heterogeneity ≥ 0.26) or between Chinese Hans and other East Asians (*P* for heterogeneity ≥ 0.14) (Supplementary Table 8). While our in silico replication was ongoing, *GLIS3* was reported as a T2D locus by the AGEN-T2D Consortium (5). The association signal (rs10814916) at *GLIS3* in our study is in high LD ($r^2 = 0.93$) with the SNP (rs7041847) identified in the AGEN-T2D meta-analysis. As such, we only consider *GRK5* and *RASGRP1* as the novel T2D loci identified by our study.

The association between *RASGRP1*-rs7403531 and T2D was confirmed by results from DIAGRAM plus GWAS meta-analysis (22) (8,130 T2D case and 38,987 control subjects of European origin), with comparable effect size and consistent direction (*P* = 0.023; OR = 1.06; *P* for heterogeneity between East Asians and Europeans = 0.22), and two imputed SNPs (rs8043085 and rs12593201, $r^2 \geq 0.8$ with rs7403531) at this locus even showed a stronger association ($P \leq 3.82 \times 10^{-3}$; OR = 1.07) in European populations from the DIAGRAM Consortium (Supplementary Tables 8 and 13). However, the T2D association for the *GRK5* locus was not replicated in the DIAGRAM plus GWAS datasets (rs10886471: *P* = 0.352, OR = 0.98; *P* for heterogeneity between East Asians and Europeans = 4.42×10^{-6}) (Supplementary Tables 8 and 13).

To examine whether adiposity was a potential mediator in the association of the two novel loci and T2D, we tested the association with further adjustment for BMI in populations across all stages. The effect sizes for each SNP remained largely unchanged (*P* adjusted for BMI = 1.73×10^{-8} to 2.87×10^{-9}; OR = 1.10–1.12) (Supplementary Table 9), suggesting that their associations with T2D were not mediated through adiposity. We further examined associations for T2D-related quantitative traits in 3,614 participants (normal fasting glucose: 2,142; impaired fasting glucose: 1,211; and T2D: 261) from the NHAPC and the GMOS studies, two population-based studies of Chinese Hans. Risk allele of the *GRK5*-rs10886471 was also associated with higher fasting plasma insulin levels (*P* = 0.0204), but not with fasting plasma glucose, whereas risk allele of the *RASGRP1*-rs7403531 was also significantly associated higher fasting plasma glucose levels (*P* = 0.0213) and lower HOMA-B (*P* = 0.0027) (Supplementary Table 11). Similar results were observed when the association analyses were performed in normal fasting glucose individuals only (Supplementary Table 11).

The *cis*-eQTL (*cis*-expression quantitative loci) analysis using the Genevar eQTL database (19) (http://www.sanger.ac.uk/resources/software/genevar/) showed suggestive evidence of association between an intronic SNP (rs4752300, $r^2 = 0.79$ with rs10886471) in *GRK5* and *GRK5* mRNA expression levels at $P = 6 \times 10^{-4}$ ($r = 0.361$ between the risk allele and *GRK5* expression levels) in adipose tissue. However, no evidence was found for associations between SNPs at *RASGRP1* and mRNA expression levels of their nearby genes. To further confirm whether rs10886471 genotypes modify *GRK5* mRNA expression, the *GRK5* expression levels were estimated using quantitative real-time RT-PCR in peripheral blood mRNA samples from 64 unrelated Chinese Hans, including 30 T2D case subjects and 34 nondiabetic control subjects. The T2D risk-increasing C-allele of rs10886471 was significantly associated with increased mRNA expression levels of *GRK5* ($P_{[dom]} = 0.02$) with the same direction of effect in both T2D case subjects and control subjects, consistent with the finding in the Genevar eQTL database. Moreover, the *GRK5* expression levels in T2D case subjects increased by 40% compared with their nondiabetic counterparts (*P* = 0.0048) (Figs. 3 and 4 and Supplementary Table 12). These results suggested that rs10886471 risk C-allele might contribute to T2D risk through increased *GRK5* expression, but should be interpreted cautiously because that blood is not a very likely tissue to be involved in T2D.

We compared the LD structure of the two novel T2D loci between Chinese Hans and Europeans and Japanese (Supplementary Figs. 6 and 7*A*), respectively. Different pairwise LD patterns were observed between Chinese Hans and Europeans at both *GRK5* (*P* = 0.0432) and *RASGRP1* (*P* = 0.0043) loci, but not between Chinese Hans and Japanese ($P \geq 0.101$) (Supplementary Table 14). The risk allele

TABLE 1
SNPs reaching genome-wide significance in a meta-analysis of combined stages 1, 2, and 3

SNP	Chrom	Position (bp)	Nearest gene	Alleles (R/A)	Stage 1*		Stage 2†		Stage 3‡		Stage 1 + 2 + 3§	
					OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
New T2D susceptibility loci												
rs10886471	10	121,139,393	*GRK5*	C/T	1.29 (1.15–1.45)	1.48E-05	1.12 (1.05–1.19)	7.71E-04	1.09 (1.04–1.15)	8.82E-04	1.12 (1.08–1.16)	7.10E-09
rs7403531	15	36,610,197	*RASGRP1*	T/C	1.21 (1.10–1.34)	1.55E-04	1.13 (1.07–1.19)	2.12E-05	1.07 (1.02–1.11)	1.78E-03	1.10 (1.06–1.13)	3.90E-09
Previously reported T2D loci												
rs2206734	6	20,802,863	*CDKAL1*	A/G	1.37 (1.25–1.51)	7.17E-11	NA	NA	NA	NA	NA	NA
rs10814916	9	4,283,150	*GLIS3*	C/A	1.15 (1.05–1.27)	3.42E-03	1.12 (1.06–1.18)	3.45E-05	1.11 (1.06–1.15)	1.98E-06	1.11 (1.08–1.15)	6.01E-12
rs2383208	9	22,122,076	*CDKN2B*	A/G	1.32 (1.20–1.46)	1.46E-08	1.19 (1.13–1.26)	5.28E-11	NA	NA	1.22 (1.17–1.28)	3.38E-17
rs11257655	10	12,347,900	*CDC123*	T/C	1.24 (1.13–1.37)	1.05E-05	1.12 (1.06–1.18)	2.22E-05	NA	NA	1.15 (1.10–1.20)	6.56E-09
rs2299620	11	2,814,871	*KCNQ1*	G/A	1.37 (1.24–1.52)	4.24E-10	NA	NA	NA	NA	NA	NA
rs4430796	17	33,172,153	*HNF1B*	G/A	1.27 (1.15–1.41)	4.83E-06	1.17 (1.10–1.24)	1.67E-07	NA	NA	1.19 (1.13–1.25)	1.52E-11
rs12010175	X	152,515,832	*FAM58A*	G/A	1.30 (1.18–1.44)	2.03E-07	1.15 (1.07–1.25)	2.49E-04	NA	NA	1.21 (1.14–1.28)	1.67E-09
rs5945326	X	152,553,116	*DUSP9*	A/G	1.28 (1.18–1.39)	7.02E-09	1.15 (1.10–1.20)	1.15E-09	NA	NA	1.18 (1.13–1.23)	6.66E-16

P values in stage 1 were corrected for genomic control before meta-analysis. Chrom, chromosome; NA, not available; R/A, risk allele/alternative allele. *Stage 1 consists of genome-wide discoveries in 1,999 case and 1,976 control subjects. †Stage 2 consists of de novo replications in 6,570 case and 6,947 control subjects. ‡Stage 3 consists of de novo replications in 3,410 case and 3,412 control subjects from SDIID/SDS and in silico replications in a meta-analysis of 6,952 case and 11,865 control subjects from the AGEN-T2D Consortium. §Stage 1 + 2 + 3 contains up to 18,931 T2D case and 24,200 control subjects in total, including 16,005 T2D case and 16,989 control subjects from Chinese Hans and 2,926 case and 7,211 control subjects from Korean, Japanese, Malay, and Filipino populations.

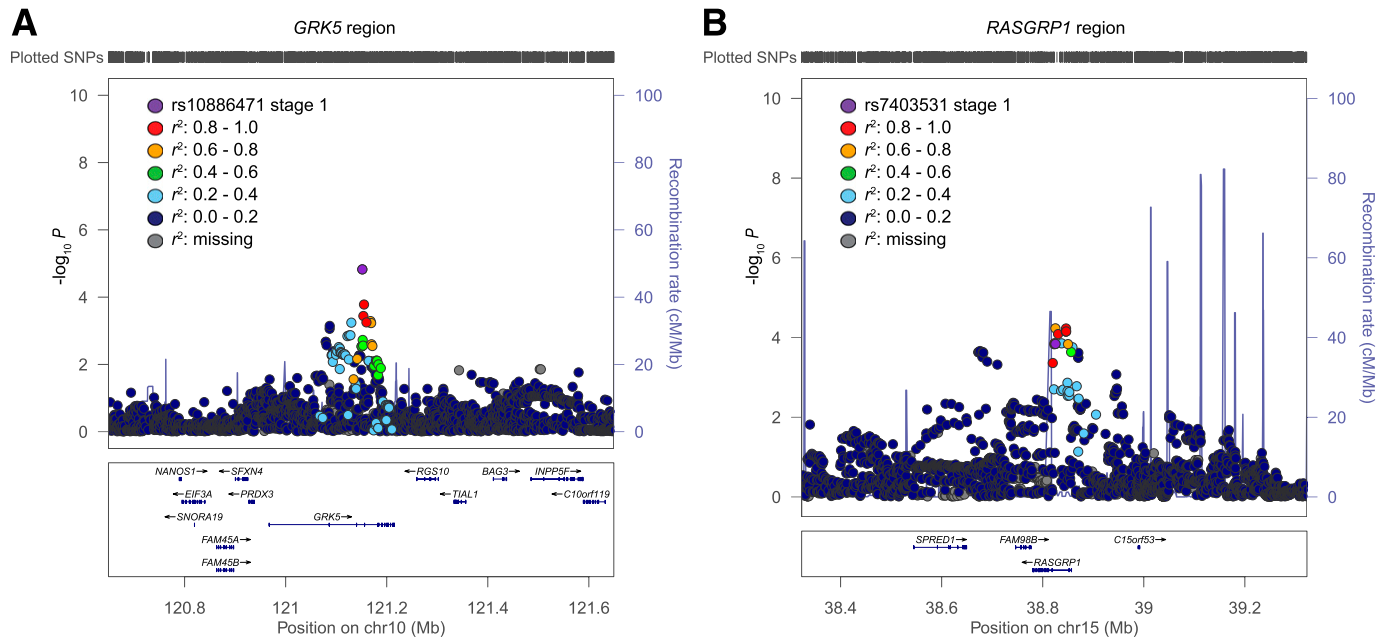

FIG. 3. Regional plots of two novel T2D loci. *A* and *B*: Imputed SNPs were estimated by MACH software (http://www.sph.umich.edu/csg/abecasis/MACH/) using LD information from 194 Asians (including 68 CHB, 25 CHS, 84 JPT, and 17 MXL) in 1000 Genome 2010·08 release as references. *P* values were from pooled analysis, adjusting for age, sex, and the first two principle components in stage 1 samples. The regional plots for the 500-kb region centered on index SNPs were generated by using LocusZoom (http://csg.sph.umich.edu/locuszoom/). The $-\log_{10} P$ values of SNPs were plotted against their genomic position (National Center for Biotechnology Information Build 37). The positions of genes were annotated from the University of California Santa Cruz Genome Browser by using GRCh37 assembly. The index SNPs are in purple. Other SNPs are colored according to their LD (r^2) with the index SNP from 1000 Genome ASN. The recombination rate is shown as a light blue line to reflect the local LD structure.

frequencies of both *GRK5*-rs10886471 and *RASGRP1*-rs7403531 in East Asians (0.79 and 0.33 in the HapMap CHB population and 0.74 and 0.45 in the HapMap JPT population for rs10886471 and rs7403531, respectively) were also higher than those in Europeans (0.48 and 0.28 for rs10886471 and rs7403531 in HapMap CEU population, respectively) (Supplementary Table 13).

DISCUSSION

In this study, we not only confirmed more than 20 previously reported loci, but also identified two novel T2D loci: *GRK5* (rs10886471) and *RASGRP1* (rs7403531). The SNP rs10886471 maps to an LD block within intron 3 of the *GRK5* gene, the only gene in this LD block (Supplementary Fig. 6). Regional plots showed that rs10886471 represents the strongest association signals at the *GRK5* locus (Fig. 3*A* and Supplementary Fig. 5). GRK5 belongs to the GPCR kinase family and plays a crucial role in phosphorylation of multiple GPCRs and non-GPCR substrates, such as glucagon receptor (31), β2-adrenergic receptor (32,33), Hsp70-interacting protein (34), and nuclear factor-κB1/p105 (35), which are either key regulators of glucose homeostasis or inflammation. Disruption of *GRK5* leads to decreased

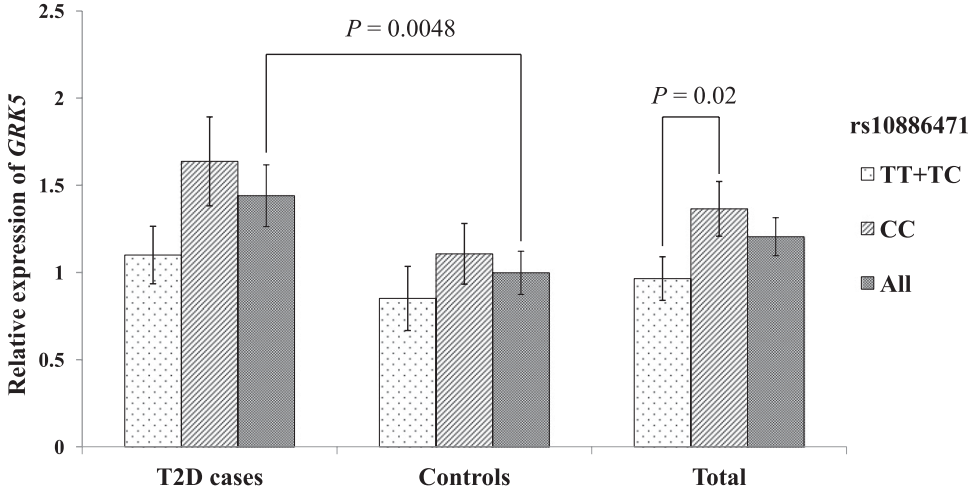

FIG. 4. Expression analysis of *GRK5*. The relative expression levels of *GRK5* were measured in blood samples from 30 T2D case subjects (rs10886471 genotype: TT+TC/*n* = 11; CC/*n* = 19) and 34 nondiabetic control subjects (rs10886471 genotype: TT+TC/*n* = 13; CC/*n* = 20 [one missing data]). Data are presented as mean and error bars (± SEM). Values of the *GRK5* relative expression levels were natural log transformed before analysis.

production of multiple inflammatory cytokines/chemokines and decreased nuclear factor-κB activation both in vivo and in vitro (36). The T2D risk-increasing allele of rs10886471 was also associated with higher *GRK5* mRNA expression levels, higher fasting insulin, but not with fasting glucose, suggesting that it might impair insulin sensitivity by increasing inflammatory response and consequently contributes to T2D risk in Chinese Hans or East Asians. Notably, *GRK5* is a population-specific T2D locus and the T2D association of *GRK5*-rs10886471 was not replicated in populations of European origin. The risk allele frequency of *GRK5*-rs10886471 in Chinese Hans was also much higher than in Europeans (0.79 and 0.48 in HapMap CHB and CEU populations, respectively), and the corresponding population attributable risk in Chinese Hans was estimated to be 8.66%. Moreover, there are significant different LD structure for *GRK5* ($P = 0.0432$) locus between Chinese Hans and Europeans.

The second novel association signal (rs7403531) is located at chr15q14 and in intron 2 of the *RASGRP1* gene (Supplementary Fig. 7*A*), which has also been replicated in Europeans from the DIAGRAM Consortium (Supplementary Tables 8 and 13). Although no heterogeneity of effect was observed between Chinese Hans and Europeans, the risk allele frequency of rs7403531 in Chinese Hans was moderately higher than in Europeans (0.33 and 0.28 in HapMap CHB and CEU populations, respectively). Regional plots showed that the strongest association signal at this locus was observed with an imputed SNP rs12593201 ($r^2 = 0.81$ with rs7403531) (Fig. 3*B* and Supplementary Fig. 5), which also showed a stronger T2D association in the DIAGRAM plus GWAS meta-analysis ($P = 2.4 \times 10^{-3}$) (Supplementary Table 13). Conditional analysis in stage 1 samples suggested that neither of the SNPs were likely to be the SNP driving the association ($P \geq 0.11$). Notably, rs7403531 was in modest LD ($r^2 = 0.46$ in HapMap CEU population) with a variant (rs7171171) previously shown to be associated with type 1 diabetes (T1D) in populations of European ancestry (37), but they are not in LD in Chinese Hans ($r^2 = 0.03$ in HapMap CHB population) (Supplementary Fig. 7*B*), suggesting that the T2D association at *RASGRP1* is unlikely to be driven by the T1D-associated SNP. *RASGRP1* encodes the RAS guanyl releasing protein 1 (RasGRP1) that functions as a guanine nucleotide exchange factor, which is required for the activation of Ras/mitogen-activated protein kinase pathways (38) and critically mediates the development and function of both T and B lymphocytes (39–42). RasGRP1-deficient mice exhibit defects in lymphocyte proliferation (40,43,44), inflammatory cytokine production (43,45,46), and apoptosis (38). *RASGRP1* is highly expressed in lymphocytes but also in various other cells, including pancreatic β-cells (47,48). Its dysfunction in β-cells may lead to islet inflammation and impaired β-cell function, which are considered as major factors involved in T2D pathogenesis (49,50). In accordance with this, the T2D risk-increasing allele of *RASGRP1*-rs7403531 was also associated higher plasma glucose and lower HOMA-B, suggesting that the T2D risk conferred by rs7403531 is likely mediated through an impaired β-cell function. However, more functional studies are required to draw a firm conclusion.

To test possible misclassification of T1D in our study, we also examined associations of all known T1D-associated variants with T2D in our stage 1 samples. In contrast to the multiple replicated T2D loci, very few of the T1D-associated variants showed a trend toward association with T2D (P values: 0.01–0.05, P for binomial test = 0.24) (Supplementary Table 15), suggesting a low misclassification rate in our stage 1 sample. Moreover, we observed a similar OR (combined $P = 1 \times 10^{-3}$; OR = 1.09) when we performed secondary association analyses for *RASGRP1*-rs7403531 in 5,678 T2D case and 8,438 control subjects from several hospital-based case-control studies, in which all T2D case subjects were negative for glutamic acid decarboxylase and insulin autoantibody tests. All of these results, together with the facts that all genotyped T2D case subjects in our study were >30 years of age and had no prior history of T1D, suggested that the observed T2D association signals at *RASGRP1* and *GRK5* were unlikely to be driven by T1D-associated variants or by case misclassification in our study.

Taken together, this study is the largest GWAS of T2D performed in Chinese Hans thus far. We have identified two novel loci (*GRK5* and *RASGRP1*) that are associated with T2D at genome-wide significant levels. In particular, the association signal at *GRK5* seems to be specific to East Asians, but this finding needs to be confirmed in further studies. We have also confirmed the known T2D loci at *KCNQ1*, *CDKAL1*, *CDKN2B*, *CDC123*, *HNF1B*, *GLIS3*, and *DUSP9* at genome-wide significant levels. Our findings not only provide new insights into the pathophysiology of T2D, but may also shed light on the ethnic differences in T2D susceptibility.

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H.L. designed the research, wrote the manuscript, and researched data. W.G., L. Lu, X.D., and Y.W. wrote the manuscript and researched data. X.H., C.H., Zh.Y., Liang S., W.B., P.L., M.H., Liangdan S., J.Z., Q.N., Y.T., R.Z., J.W., D.W., X. Zhu, K.G., X.Zu., X.G., H.Y., X.Zho., DIAGRAM Consortium, AGEN-T2D Consortium, X.Zha., T.W., Ze Y., and W.J. researched data. L.Q., R.J.F.L., and F.B.H. contributed to discussion and reviewed and edited the manuscript. Y. Liu, L.Li., R.H., L.J., and Y. Li designed the research, researched data, and contributed to discussion. X.L. contributed to project design, management, and coordination; wrote the manuscript; and researched data. All authors reviewed the manuscript. X.L., Y. Li, and L.J. are the guarantors of this work, and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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